EFFECTS OF WEIGHTLESSNESS ON THE EMBRYONIC DEVELOPMENT AND AGING OF DROSOPHILA

J. Miquel

D. E. Philpott

P. R. Lundgren

R. Binnard

C. E. Turnbill

NASA-Ames Research Center

Moffett Field, California 94035

A B S T R A C T

The biological effects of weightlessness have been investigated on Drosophila melanogaster of the Domodedov-32 strain, which developed and spent the first days of adult life in space. Following a 19.5 day exposure to zero g, the flies were studied by morphological, chemical and behavioral techniques. Apparently, the development of Drosophila was insensitive to weightlessness and the aging process was not influenced, except for a slight reduction in the amount of lipofuscin present in the midgut and Malpighian tubules.

KEY WORDS: Weightlessness, Drosophila, Embryonic development, Aging.

INTRODUCTION

All living organisms have evolved under the influence of the gravitational field of the earth, and, therefore, it is generally assumed that gravity has played a role in shaping their structure and function (2). This, however, could not be experimentally confirmed until the advent of the Space Age. Even a biologist of the scientific stature of Darwin, commented that we would never be able to escape gravity and perforce would remain ignorant concerning its role in evolution and development (14). About one hundred years after Darwin, the space programs of the USSR and the USA have made space travel possible and numerous observations have documented the important physiological effects of weightlessness on human subjects (12, 13, 20). On the other hand, space flight experiments suggest that zero g does not influence in a drastic manner fundamental biological processes such as embryogenesis and growth, at least as regards insects. In effect, the Tribolium experiment performed as a part of the Biosatellite II program failed to demonstrate any effect of weightlessness on the development of this insect (1). This is in agreement with numerous observations performed on another insect species, namely Drosophila melanogaster, which has been exposed to zero g in Soviet manned vehicles and unmanned biosatellites. As stated in a review of USSR space biology, published in 1968 (24): "Copulation, oviposition and normal development of Drosophila can occur normally during periods of weightlessness up to four days. Embryonic development of a normal strain of Drosophila under weightless conditions does not result in statistically significant increases in morphological changes or mutations in sex cells. In these experiments on "Vostoks 3 and 4", however, the number of females in all cultures considerably exceeded the number of males, and the latter hatched somewhat latter".

More recently, the Soviet space biologists showed that Drosophila exposed to weightlessness during a 22-day "Kosmos" flight could complete nearly two entire developmental cycles from fertilization to hatching. Therefore, the

conclusion was reached that "...weightlessness or even any other factors of space flight could not have affected the duration of Drosophila development" (19).

Previous work from our laboratory has documented that the aging process of Drosophila is strikingly sensitive to altered environments, including temperature changes (16) and rotation in a clinostat (16). Moreover, data from Biosatellite II show an apparent increase in the life span of Habrobracon wasps which were exposed to zero g (22). This, in our opinion, made advisable to extend the research on the effects of weightlessness to the last stages of the insect life cycle, i. e. the adult period, including senescence. Therefore, the present work focuses in the aging process of fruit flies. which developed and spent their first days of adult life in a "Cosmos" biosatellite.

METHODS

The flies used in this study were wild Drosophila melanogaster of the Domodedov-32 strain. They were placed in the Soviet biosatellite as larval cultures, in vials containing standard medium. At launch, the flies were 4 days old, as estimated from oviposition time. The experimental Drosophila population was exposed to weightlessness during about 19.5 days, and an equal number of flies, which were housed in a centrifuge at approximately 1 g aboard the "Cosmos" vehicle, served as flight controls. Two other groups, namely "synchronous" and "laboratory" controls were part of the experiment. The synchronous flies were kept in a mock-up of the biosatellite, on the ground, throughout the time of the space flight. The laboratory controls were Drosophila maintained on the ground under standard laboratory conditions.

During space flight in the biosatellite the flies were exposed to the following environmental conditions: 742-850 mm Hg of total atmospheric pressure; pO_2 =140-253 mm Hg; pCO_2=2.3-8.0 mm Hg; temperature=19-21.3°C; relative humidity=40-62 %. The flies were maintained in total darkness for the entire duration of the flight.

Additional data on the experimental plan and flight factors can be found in the introductory article on the "Cosmos-75" flight.

Table 1 shows the number of Drosophila which were delivered to the Ames laboratory in satisfactory condition, at 28-29 days after launch and 7-8 days after landing of the biosatellite. Total exposure of the experimental Drosophila to weightlessness was approximately 19.5 days. Since hatching was expected to occur on the 7 or 8th day after launch, adult Drosophila were only exposed to weightlessness during a period of 12 to 13 days.

Upon arrival at the Ames laboratory all wild flies were individually weighed in a Cahn electric balance. Then, they were transferred to vials containing fresh food and placed in incubators at 21°C, under continuous light of moderate intensity. Humidity was approximately 40 %. Transfer to fresh food vials was performed once a week, as in previous work from our laboratory (3,4,6,7,8,10,15).

The following parameters were investigated on experimental and control flies:

1) Body weight, which was determined on all flies, following etherization.
2) Vitality, as expressed by negative geotaxis and mating. Negative geotaxis is measured using a glass-stoppered volumetric cylinder of 250 ml fitted with a layer of soft plastic on its bottom. When shaken to the bottom by tapping the cylinder against a rubber mat, the flies quickly run or fly to the upper region. From 20 to 50 flies are used in each measurement, and the results are expressed as the percent of flies which cross the 250 ml mark in 20 seconds. Mating competence was determined as in previous research (4). Individual males were placed in empty vials (15 cm in length and 2.5 cm in diameter). Then, three 7-day-old virgin females were placed in each tube,

Table 1: Number of experimental and control wild Drosophila melanogaster of the Domodedov-32 strain used in this investigation.

	♂ Number	♀ Number
Experimental (exposed to weightlessness)	101	100
Flight controls (maintained in a centrifuge at~1g)	91	97
Synchronous ground controls	52	46
Laboratory ground controls	43	48

a cotton plug was pushed half-way into the tube and the mating behavior of the flies was observed for one hour. The time to the first mating (mating latency) and the number of matings in one hour were recorded for each of the male flies tested. At the end of one hour, males and females were separated and saved for future studies, including quantitative determination of the offspring produced.

3) External morphology, as demonstrated by gross photography and scanning electron microscopy of control and experimental flies.

4) Age-associated degenerative changes, investigated by light and electron microscopy using the techniques described in our previous publications (6,7). Ten flies from each group were used for these morphological studies.

5) Glycogen content of the thorax. This parameter was investigated by freezing the flies in liquid nitrogen, separating the thorax from the head and the abdomen, and analyzing the glycogen by the glucose oxidase technique, following extraction with KOH and ethanol precipitation. Since the Drosophila thorax is packed with wing muscles, the glycogen analysis provides a good estimate of the energy reserve of the muscles which support the insect flight.

6) Life span, was determined in a sample of each group of male and female space-flown Drosophila. Since the time of hatching was not known with exactitude, all ages in the present article are given in days since the laying of the eggs from which the imagoes used originated.

RESULTS

The mean body weights for all Drosophila populations, at 33 days of age, are shown in Table 2.

Table 3 summarizes the results of the analysis of thorax glycogen and Tables 4 and 5 give the values obtained for negative geotaxis and mating competence of experimental and control flies.

Various aspects of the external morphology, which was normal for all Drosophila,

Table 2: Body weights of flies exposed to weightlessness and controls, at 33 days of age.

Number of flies	Males	Mean body weight (in mg) ± S. E.	
43	Laboratory controls	0.948	0.011
52	Synchronous controls	1.098	0.009
91	Space-flown at ~1g	1.060	0.012
101	Space-flown at ~zero g	1.067	0.009
	Females		
48	Laboratory controls	1.354	0.016
46	Synchronous controls	1.527	0.023
97	Space-flown at ~1g	1.585	0.019
100	Space-flown at ~zero g	1.588	0.015

Note: The space-flown male *Drosophila* are lighter than synchronous controls and heavier than laboratory controls ($p = 0.030$ and $p < 0.001$, respectively).

Table 3: Glycogen content of the thorax of 48 day old flies. (The analysis was performed on samples containing two isolated thoraces).

Number of samples		μg of glycogen per 2 thoraces: mean values ± S. E.	
5	Synchronous controls	37.9	1.6
5	Space-flown at ~1 g	35.9	1.1
5	Space-flown at ~zero g	34.6	1.6

Table 4: Negative geotaxis of control and experimental male flies, at 65 days of age.

	Percent of flies able to reach the 250 ml mark in a volumetric cylinder in 20 seconds (mean of 10 consecutive trials).
Synchronous controls	85
Space-flown at ~1 g	73
Space-flown at ~zero g	78

Table 5: Mating competence of male flies tested at 62 days of age. (Every male used in this experiment was kept in a test tube with 3 young virgin females of our control D. melanogaster Domodedov-32 population. The time to the first mating (latency) and the total number of matings in 1 hour were recorded for each male fly tested).

Number of males		Total no. of first matings	Total no. of 2nd matings	Average no. matings/♂	Latency (min.)
20	Synchronous controls	20	16	1.5	6.6
20	Space-flown at ~1 g	15	7	1.1	12.1
20	Space-flown at ~zero g	19	8	1.3	11.6

including the population exposed to weightlessness, are illustrated in Figures 1 to 8.

As shown by Figures 9 and 10, the fine structure of the wing muscle of flies exposed to space weightlessness was perfectly normal. Moreover, the fat body, oenocytes (Figure 11) and testes (Figure 12) could not be distinguished on the basis of their fine structure from the same organs of the flight controls.

As in old Drosophila melanogaster Oregon R from our laboratory (6,10,17), lipofuscin accumulation was very striking in the oenocytes of old D.melanogaster Domodedov-32, including the flies exposed to weightlessness (Figure 11). Moreover, the accumulations of intramitochondrial crystals and virus-like particles first observed by us in D. melanogaster Oregon R, were also noticed in the Domodedov strain (Figures 13 and 14).

Seemingly, the only fine structural difference between Drosophila exposed to weightlessness and the flight controls was a somewhat better preservation of the cells of the midgut and of the Malpighian tubules. The dense body accumulation characteristic of the cells of old Drosophila (Figures 15 and 16) was not so evident in the flies which were exposed to zero g.(Figure 11). However, this effect was not accompanied by an increased longevity, since as shown in Table 6, the life span of the Drosophila exposed to weightlessness was not significantly increased as compared to that of the control populations.

DISCUSSION

Our present observations are in agreement with previous research by Antipov and Parfenov (cited by Wukelic, 24) and with more recent data by Parfenov (cited by Dubinin and Vaulina, 2) showing that weightlessness does not seriously interfere with Drosophila development. An apparent lack of striking effects of zero g on the embryonic development of amphibians (25) and fish (21) has also been documented by American space biologists.

It is conceivable that prolonged exposure to weightlessness may induce wing

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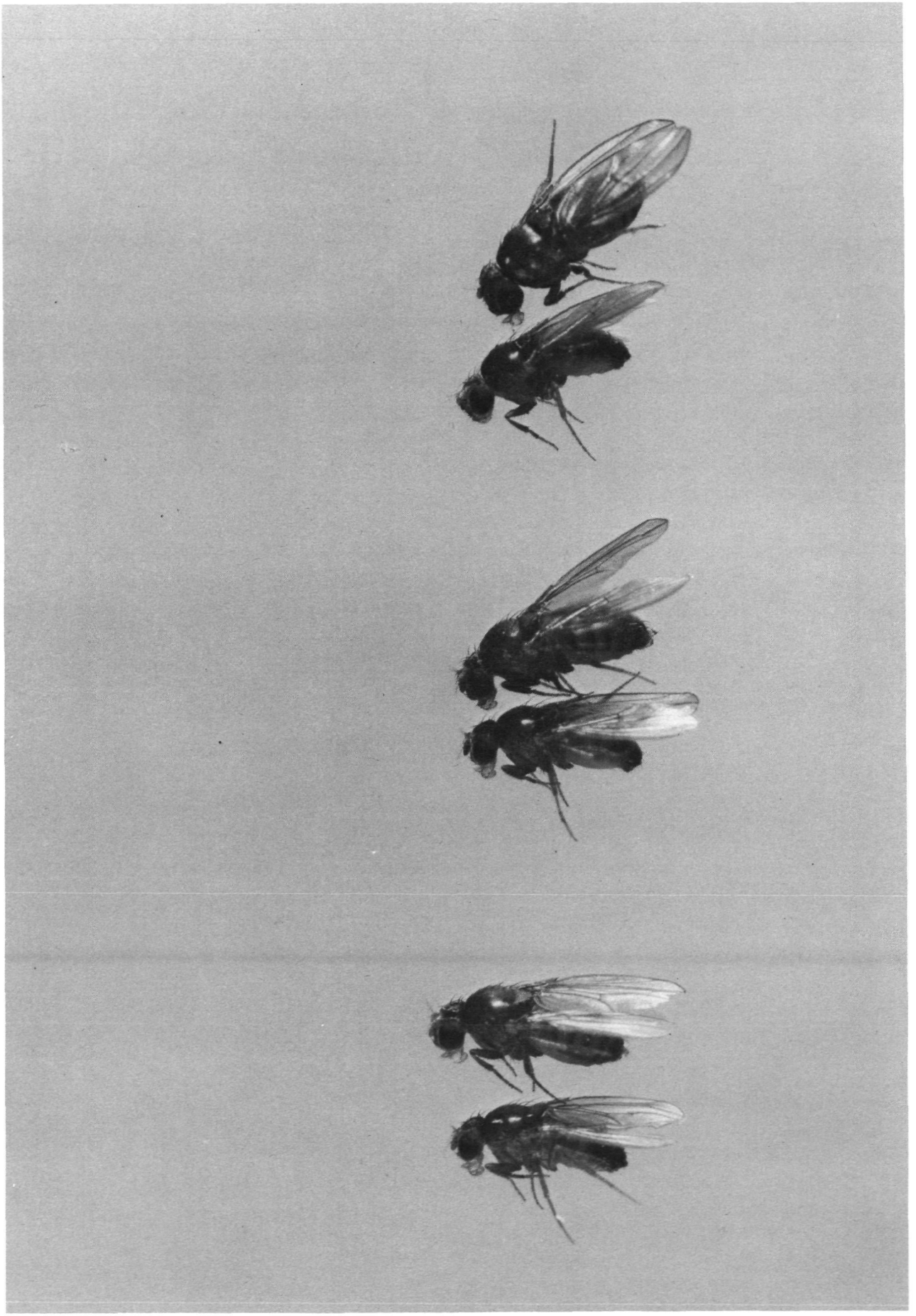

Fig. 1. Synchronous ground controls and space-flown Drosophila. Approximately X10. Right male and female couple: synchronous controls. Middle couple: flies which were exposed to weightlessness aboard the Cosmos biosatellite. Left couple: flight controls maintained in a centrifuge at about 1 g in the biosatellite.

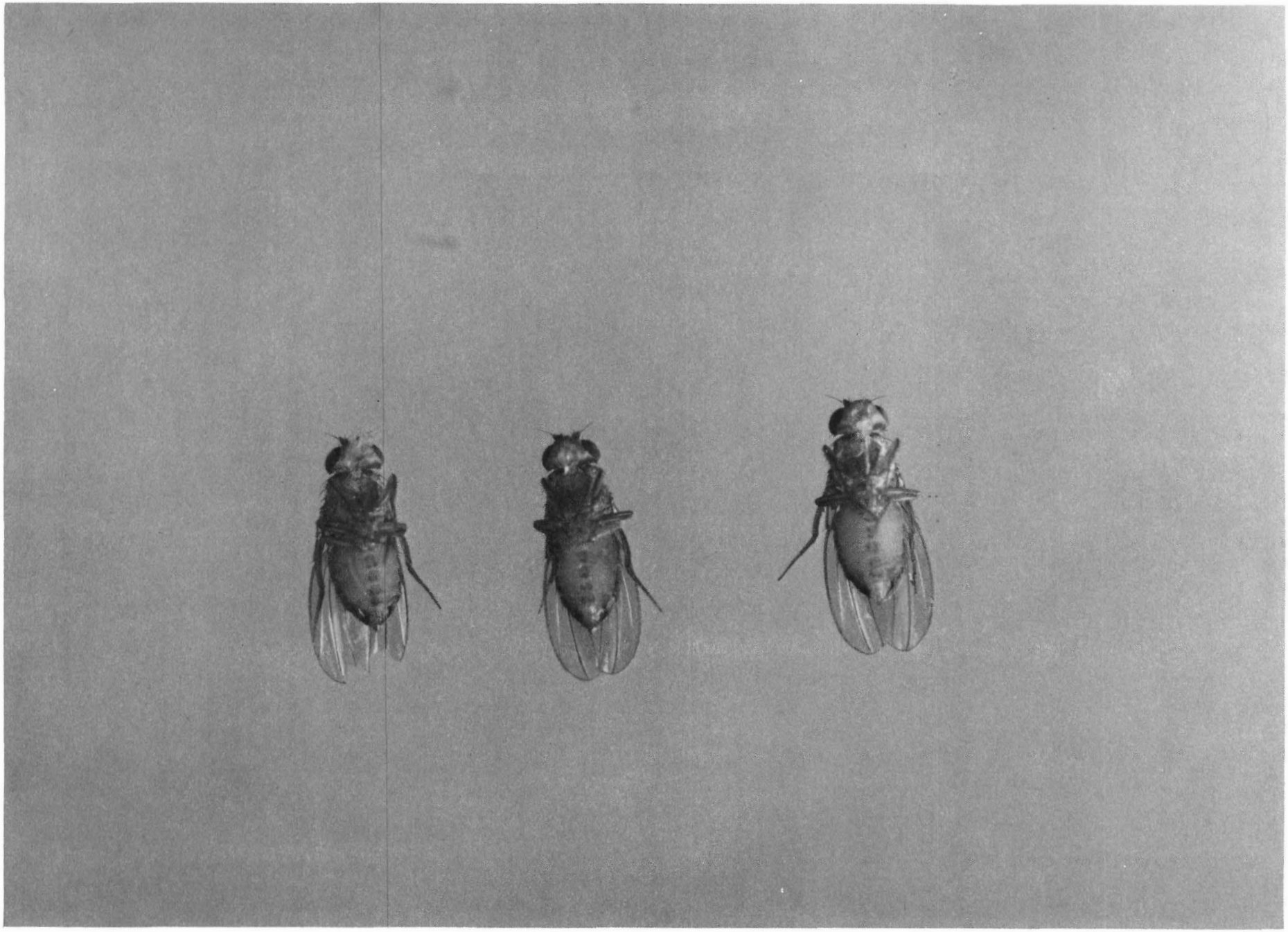

Fig. 2. Synchronous ground controls and space-flown Drosophila. Approximately X10. Ventral view of flies arrange in the same order as in Fig. 1.

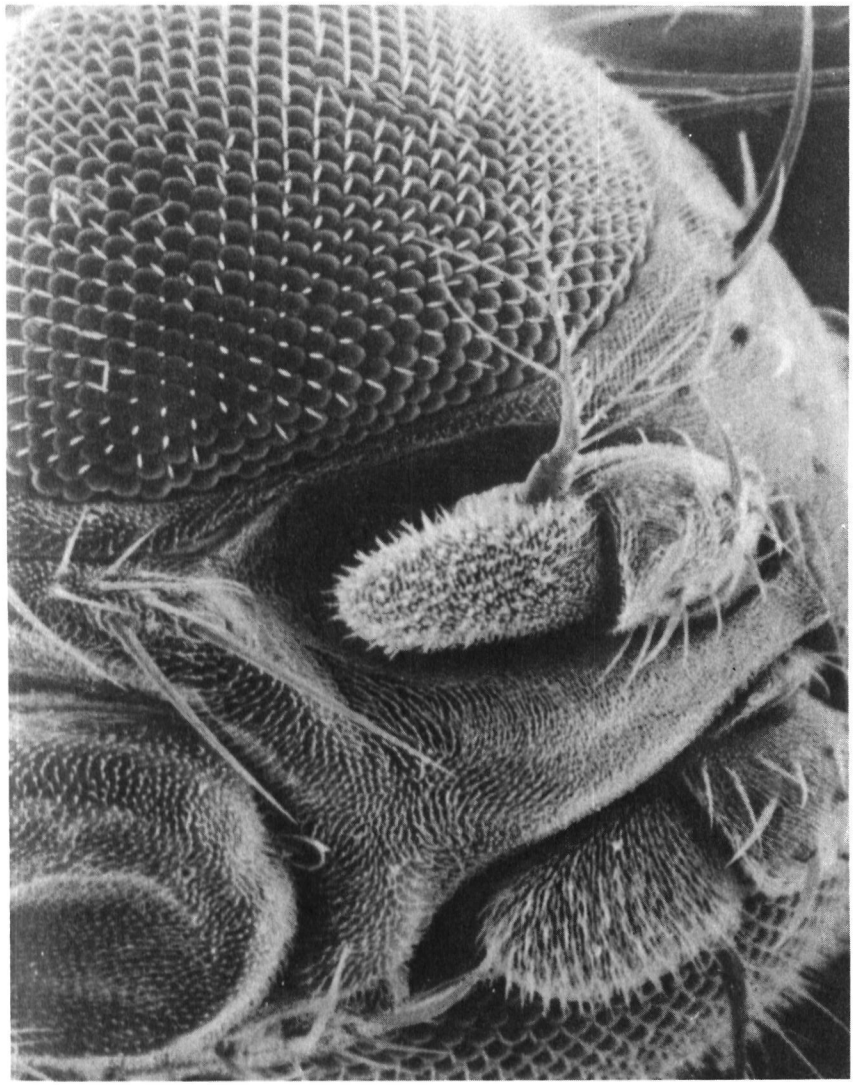

Fig. 4. Drosophila flown in a centrifuge aboard the biosatellite. External morphology of the head. X570.

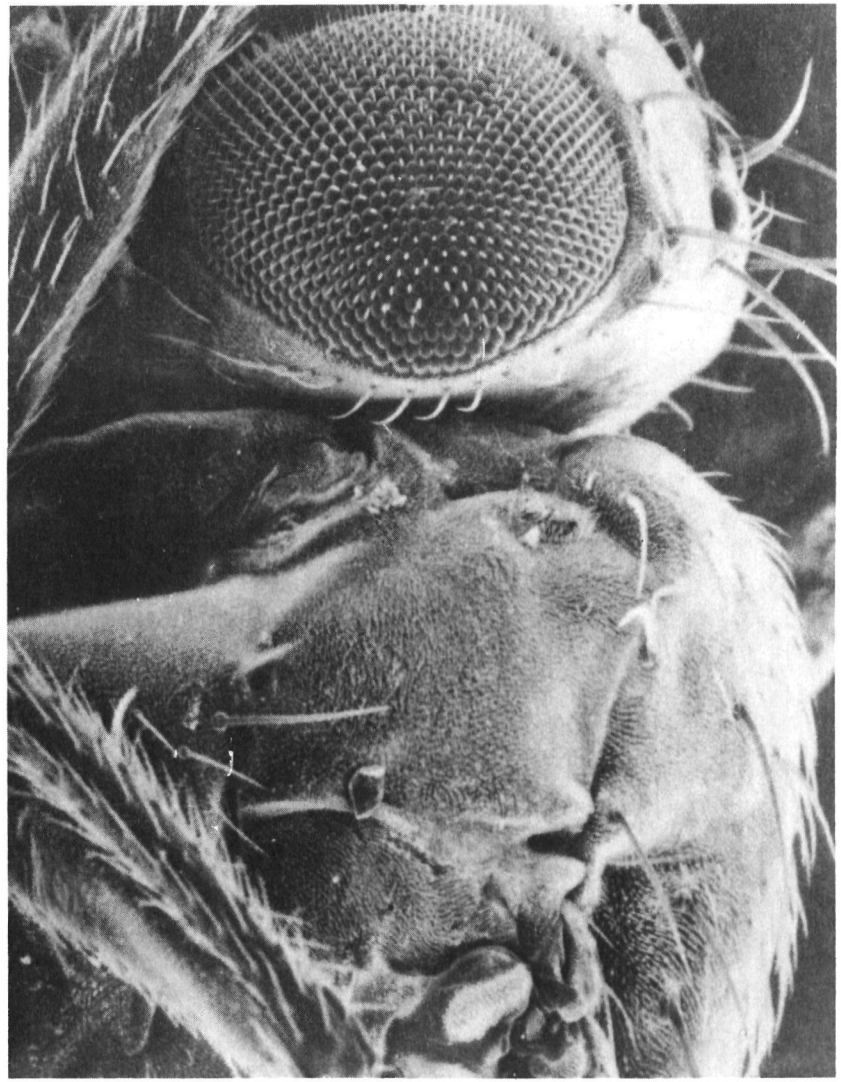

Fig. 3. Drosophila flown in a centrifuge aboard the biosatellite. Side view of the head and thorax. X390

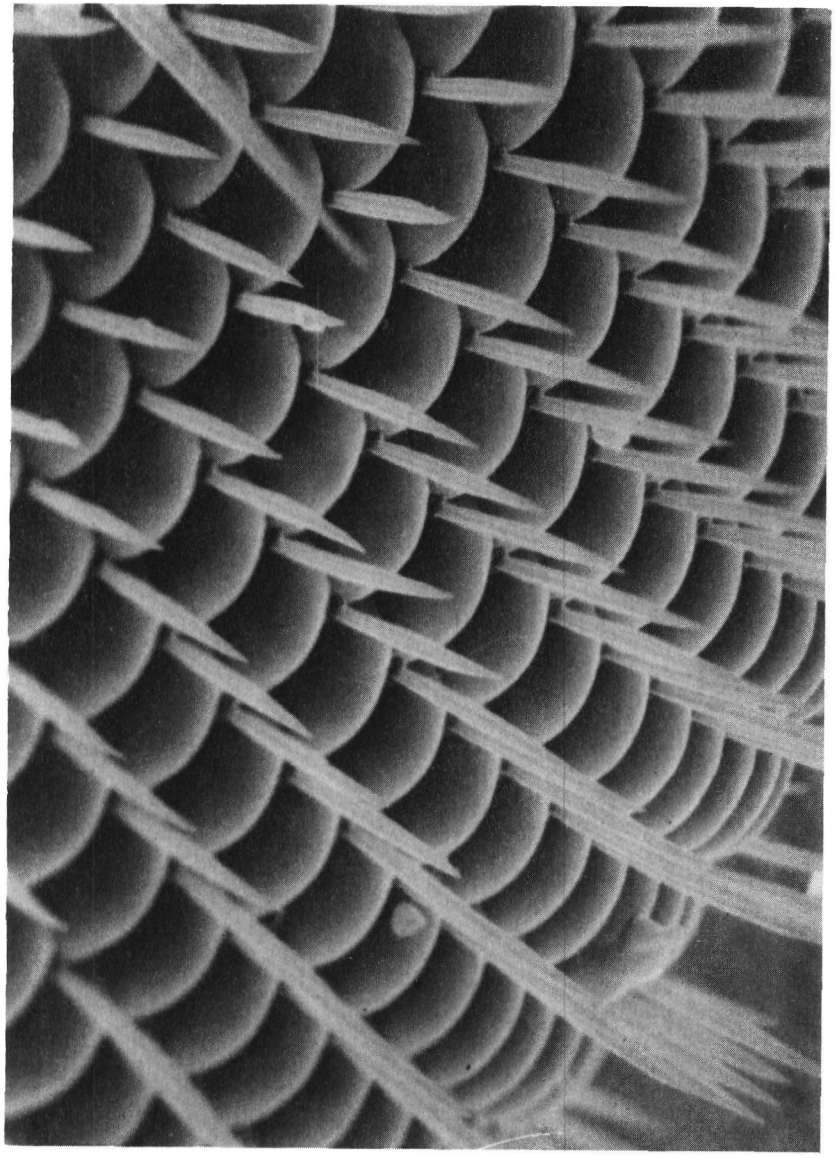

Fig. 6. Drosophila flown in a centrifuge aboard the biosatellite. High magnification scanning electron micrograph of the lenses and setae. X3000.

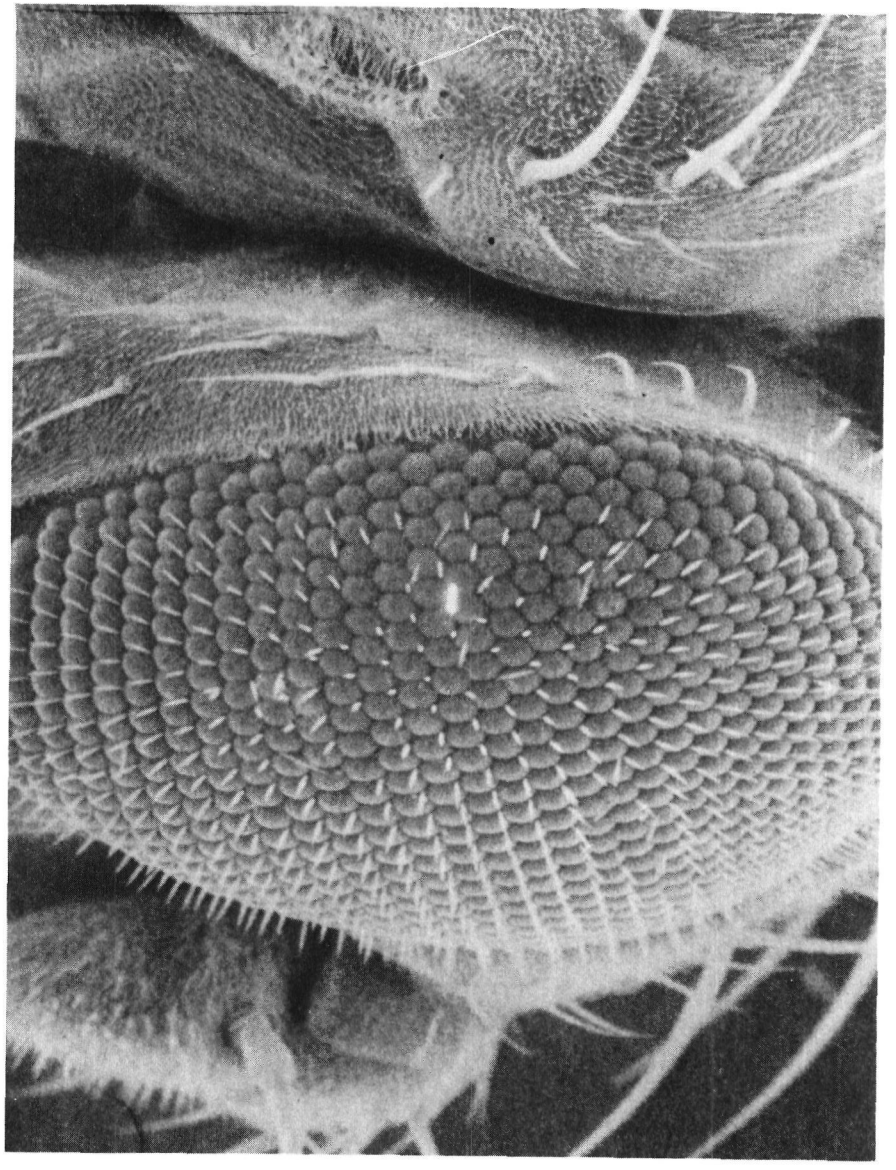

Fig. 5. Drosophila flown in a centrifuge aboard the biosatellite. Eye, showing the lenses and setae. X500.

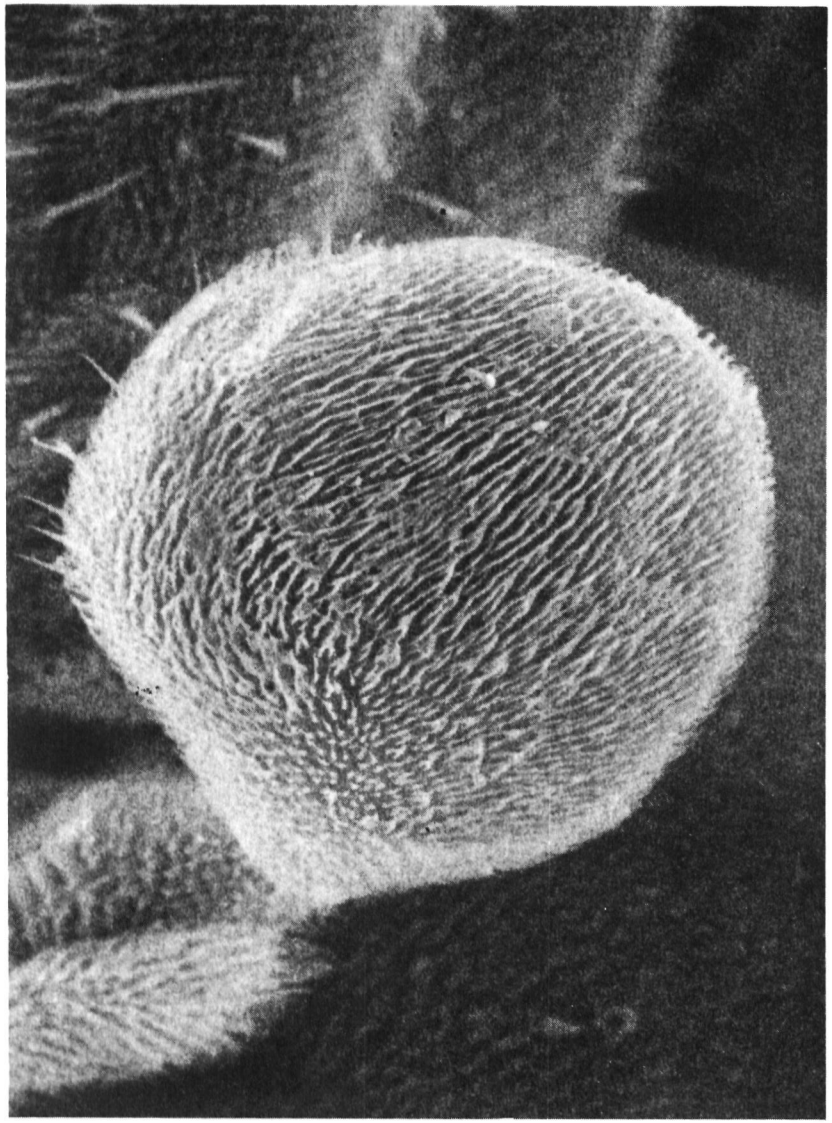

Fig. 8. Drosophila flown in a centrifuge aboard the biosatellite. Haltere (equilibrium organ). X500.

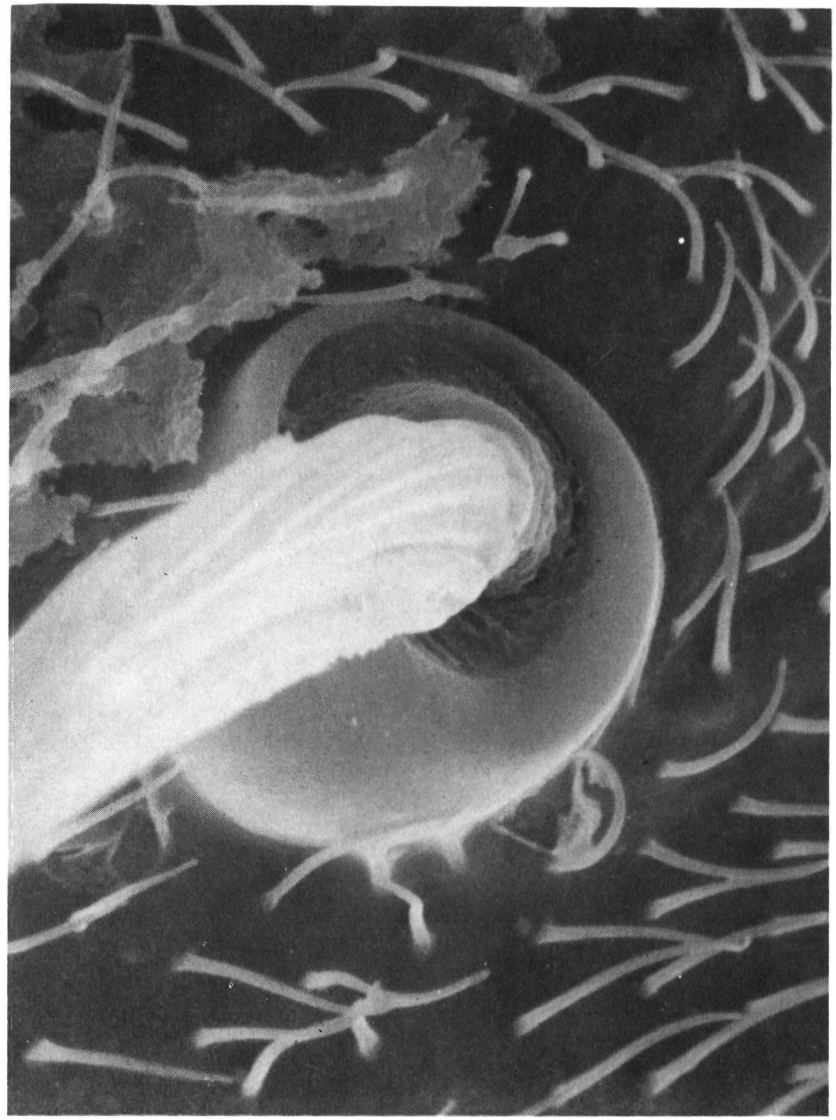

Fig. 7. Drosophila flown in a centrifuge aboard the biosatellite. Norphology of one of the vertical setae of the head. X2000.

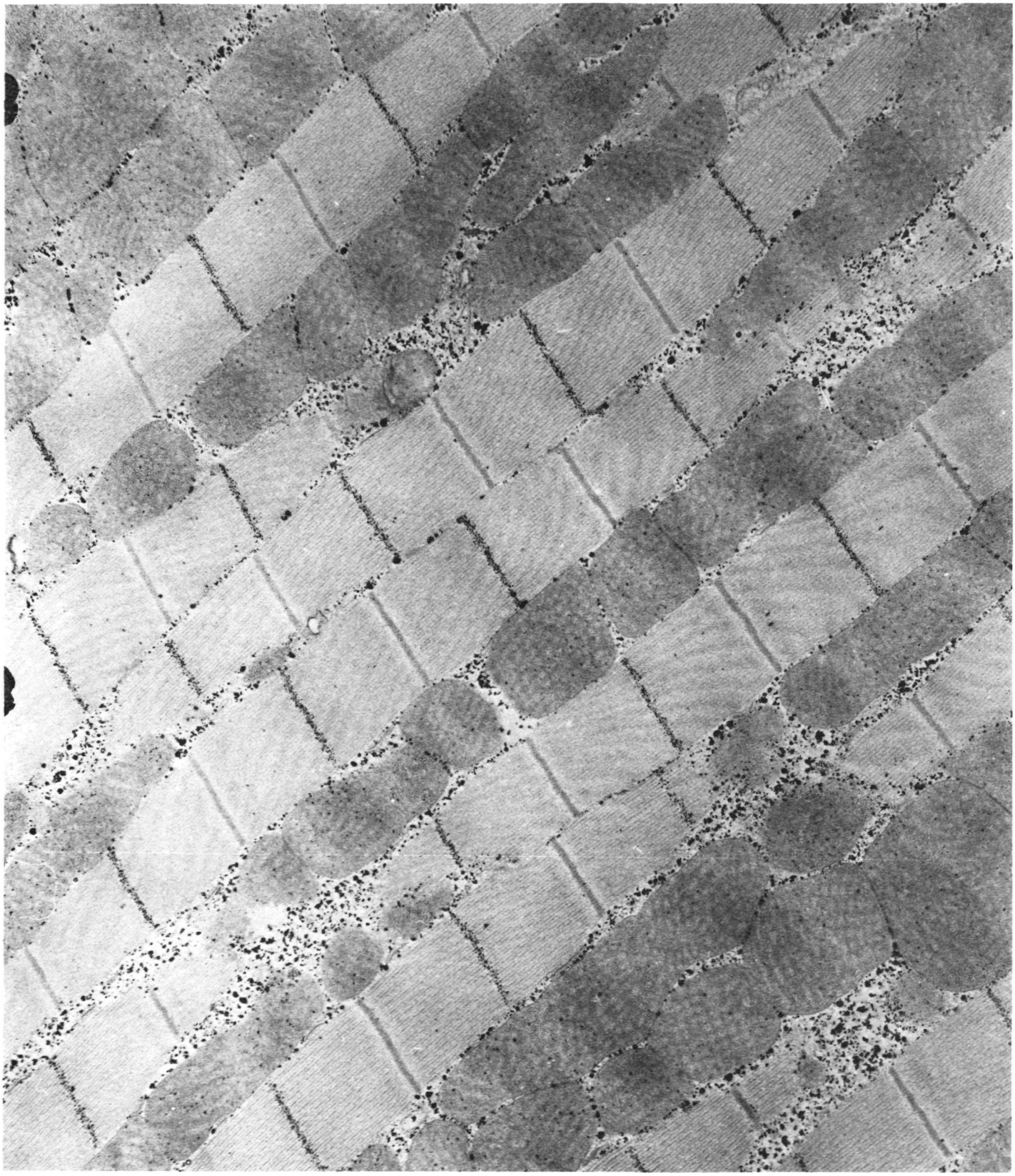

Fig. 9. Wing muscle of a 110 day old fly which was exposed to weightlessness, showing a normal appearance of myofibrils, mitochondria and glycogen granules. X11, 375.

Fig. 10. High magnification electron micrograph of 110 day old Drosophila exposed to weightlessness. No atrophic changes are present. X25,650.

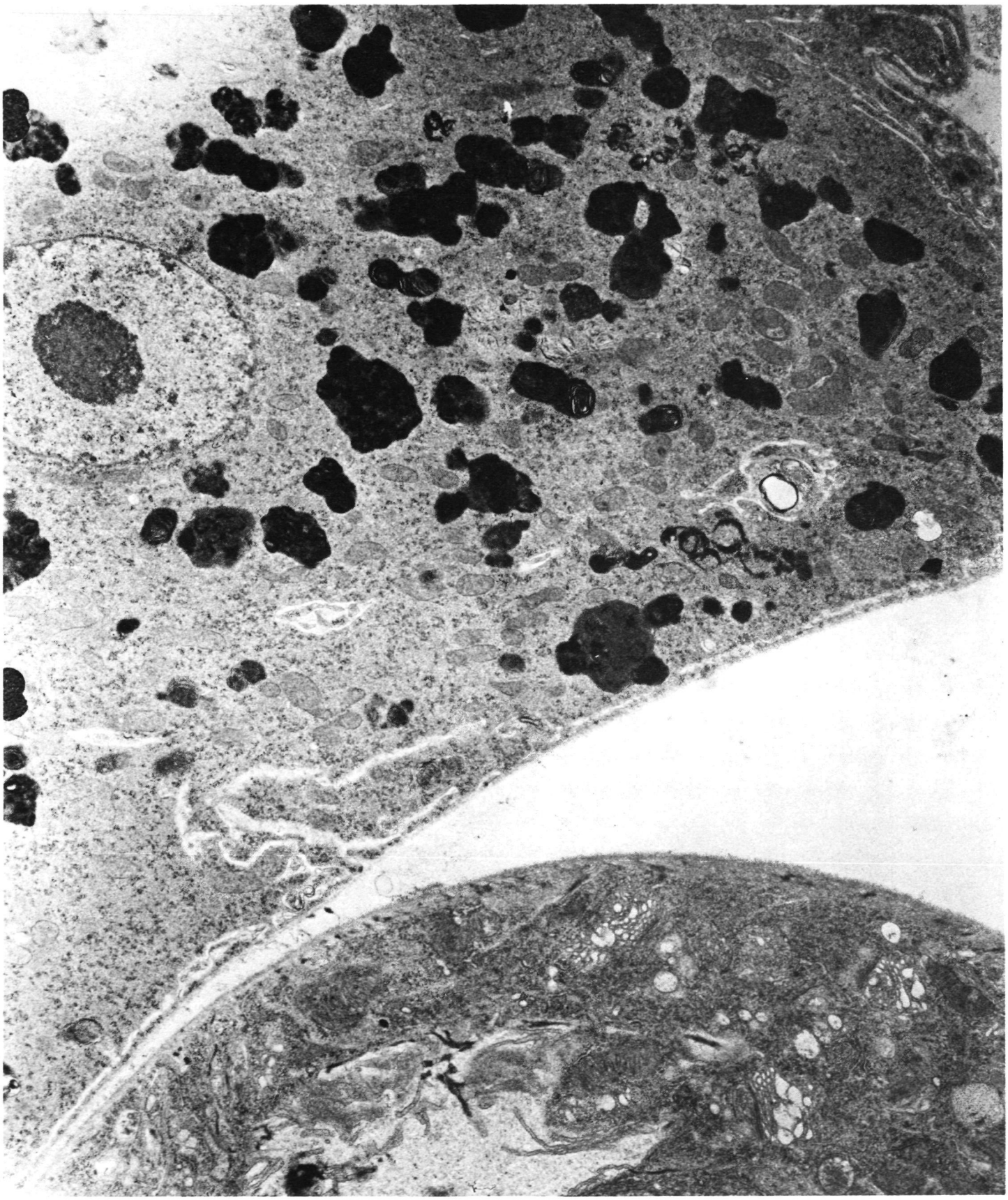

Fig. 11. Oenocytes with abundant dense bodies (age pigment) and a Malpighian tubule (lower area) in the abdomen of a 110 day old fly which was exposed to weightlessness. X11,375.

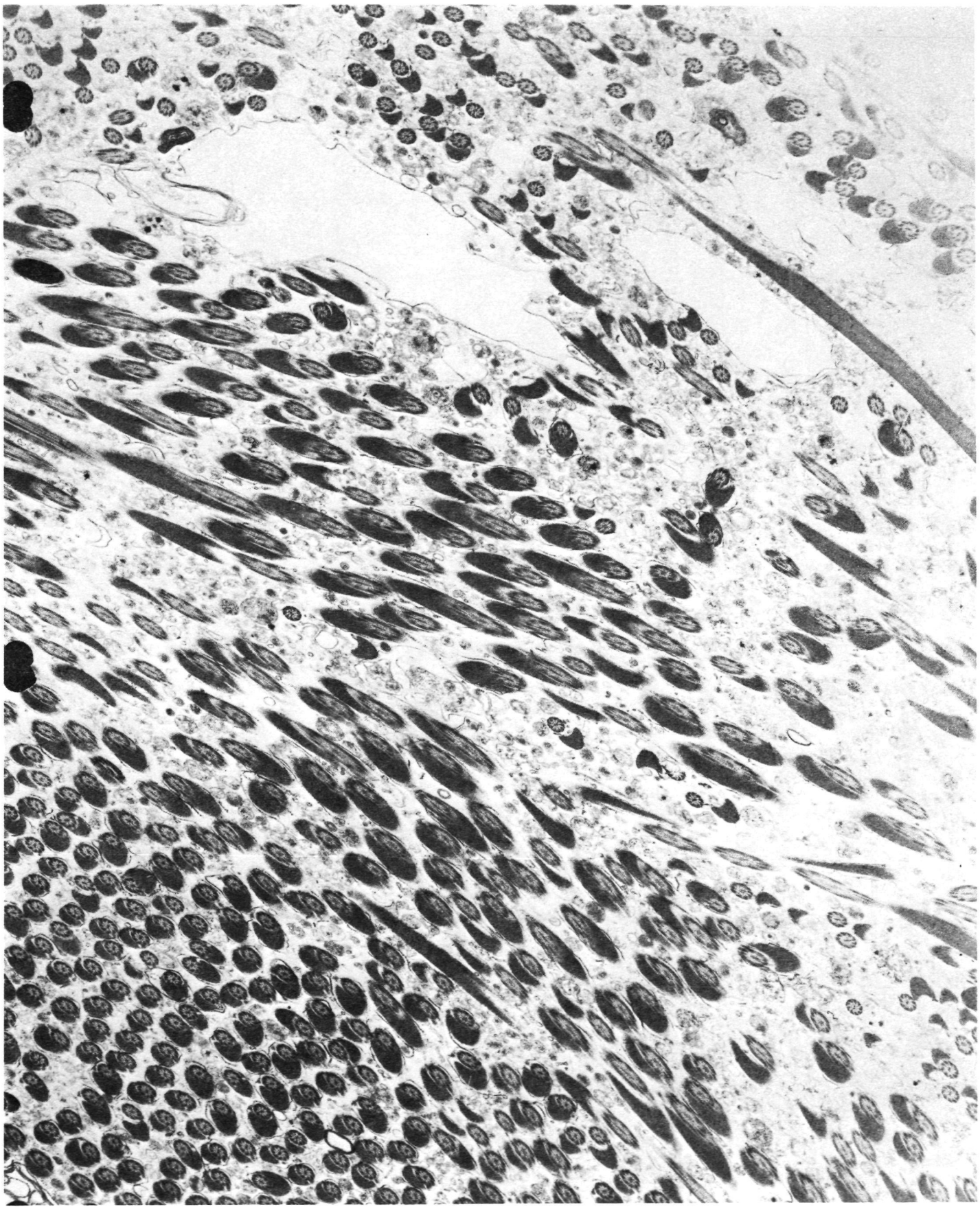

Fig. 12. Spermatozoa tails in the testes of a 110 day old fly which was exposed to weightlessness. X11,375.

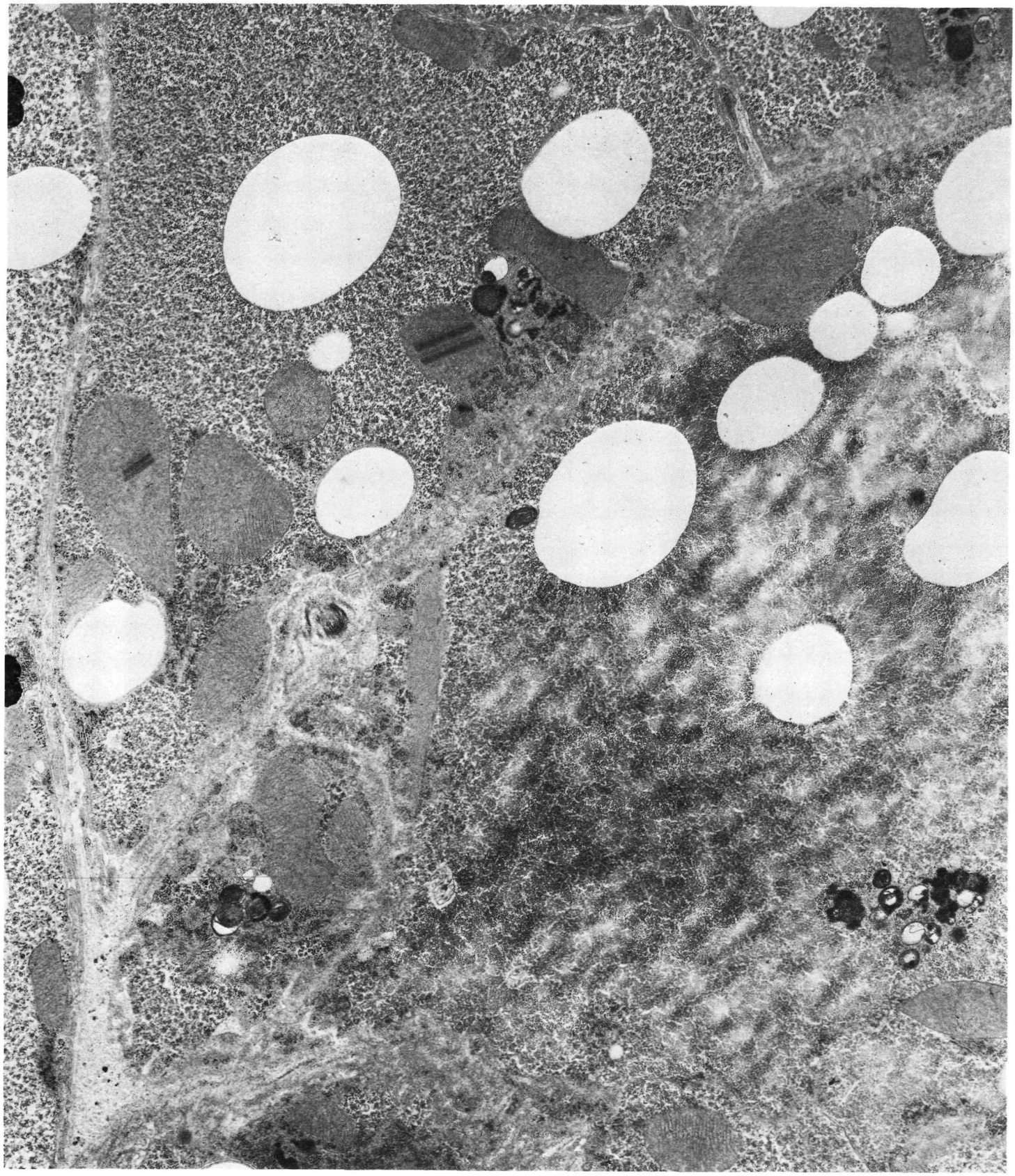

Fig. 13. Fat body cells from the abdomen of a 110 day old fly which was maintained in a centrifuge aboard the biosatellite. Lipofuscin granules, glycogen and two mitochondria containing crystalloid structures can be seen. X11,375.

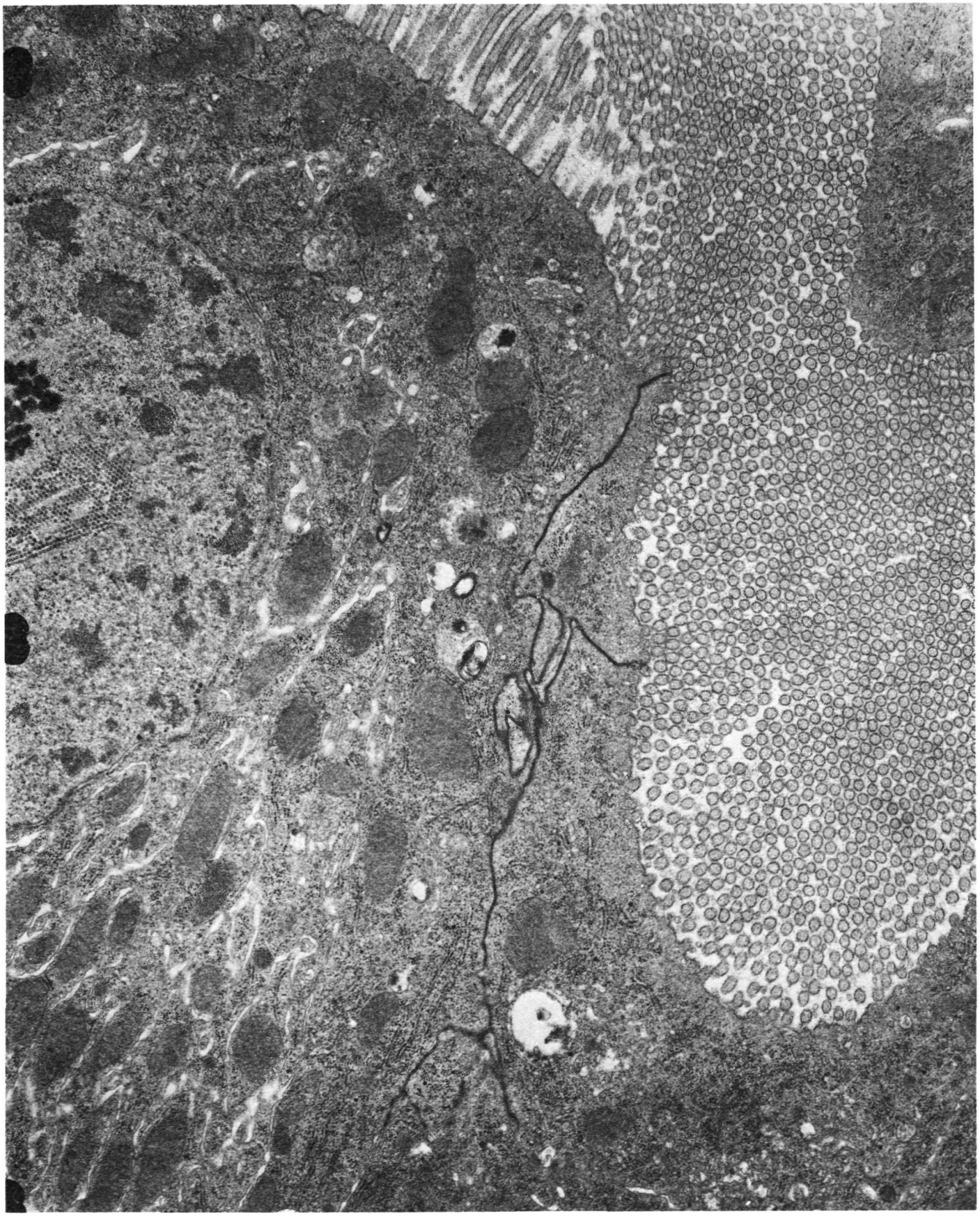

Fig. 14. Virus-like particles in the nucleus (upper left) of a midgut cell of a 110 day old flight control. X17,000.

Fig. 15. Fat body cells (left) and midgut (right) containing lipofuscin granules. The amount of pigment in the tissues of this 110 day old flight control is approximately the same than in D. melanogaster Oregon R of similar age. X11,375.

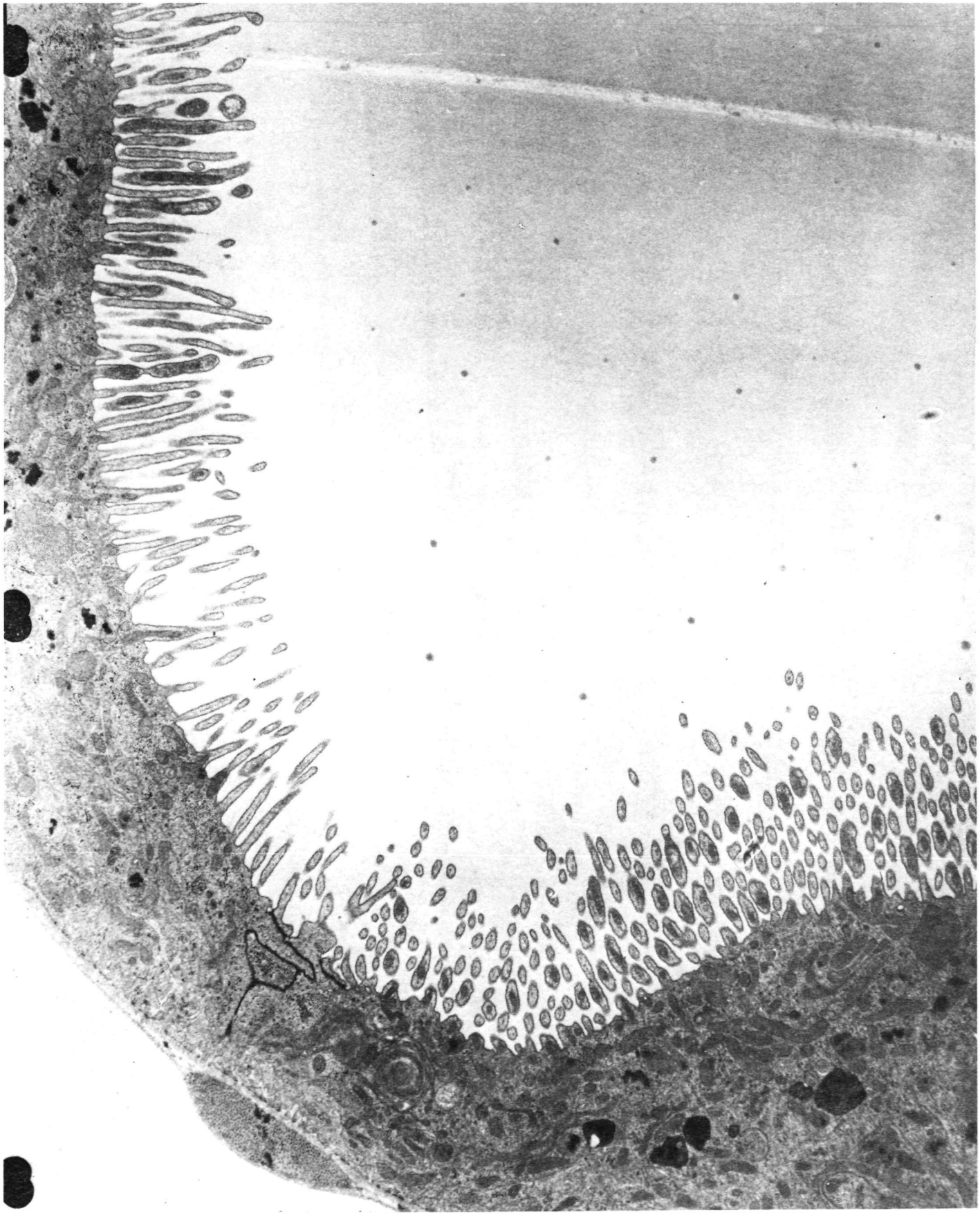

Fig. 16. Lipofuscin granules in the Malpighian tubules of a 110 day old flight control. X11,375.

Table 6: Life span of "Kosmos"-flown Drosophila

	♂			♀		
	Number	Mean life span (days)	± S.E.	Number	Mean life span (days)	± S.E.
Maintained at ~1 g (centrifuge)	31	102.6	5.4	49	109.7	3.7
Exposed to zero g	29	105.2	6.6	61	105.0	2.7

muscle atrophy, if effective flying is rendered difficult or impossible in the absence of gravity. However, our observations on glycogen content and muscle fine structure have not revealed changes similar to those described by Ilyin et al. (5) in their report on the effects of zero g on the leg muscles of rats exposed to weightlessness.

As regards the aging process, our data do not show any significant difference between the flies exposed to weightlessness and the flight controls, except for a slight reduction in the amount of age pigment of the midgut and the Malpighian tubules.

A comparison of the space-flown Drosophila with the ground controls documents the fact that the flies resisted extremely well the stresses of the "Cosmos" flight. The only detrimental effect of the flight seemed to be a decrement in the negative geotaxis and mating competence. This was particularly evident for the mating latency, that was almost double for the "Cosmos" flies than for the ground synchronous controls. It is likely that this decreased mating ability was the result of injury to the wing structures (which play a crucial role in Drosophila courtship), as the consequence of the accelerations or of other stresses unrelated to weightlessness.

In summary, our data provide very conclusive evidence about the lack of effects of weightlessness on insect development, since the flies exposed to zero g were practically identical to the flight controls as regards every parameter investigated. With respect to the possible influence of weightlessness on the aging process, we can not be so definitive. It seems to us that adult Drosophila should spend in weightlessness at least one third of its normal life span, in order to provide more conclusive evidence on this problem than has been possible to obtain by this preliminary research.

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